

15 Grow 200 ml Buffered rich medium (Fermentation) with 1 ml of o/n grown cells

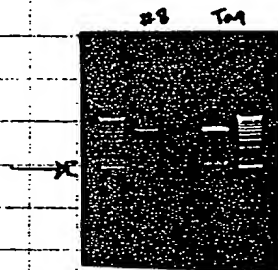
Both #1 & #3 were grown

Grow at 28°C (the temp. of the incubator) until A₅₉₀ ~ 0.7
 Induce with IPTG (1 mM) for 3 hrs → harvest.

Give cells to Adam Goldstein for purification/enzyme assay.

DH10B background

667Y Digest #8 with NgoAIV + XbaI in Reed 1. } As before
 Digest PTQ18-Tag with " " " " }



Purify both vector (Tag) plus the mutant fragment by gene clean as before.

Ligation: 15 µl DNA mix
 4 µl 5X buffer
 1 µl T4 ligase

Ligate 2 hrs / room temp

Transform DH5α mcr⁺ rec⁺ with 2 µl ligation.

Incubate plates at 30°C.

DH5α mcr⁺ rec⁺
background

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Date

Invented by

Date

Recorded by Baltes

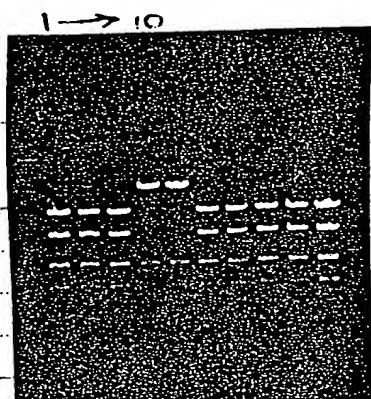
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10% plating 154 colonies
 90% plating >800

Inoculate 10 colonies for mini prep tomorrow. 2ml LB/amp.
 30°C.

Standard miniprep from 1ml culture

DNA was dissolved in 150µl TE
 Digest with Ase I (17µl DNA in NEB react 3)



Except for #4 & 5 all clones contains an extra Ase I site (the largest band was cut with an extra Ase I site that created in the oligo)

Thus, pool all plasmids (except 4 & 5)

Save cultures #1, 2 and #3.

Grow #1 and #2 for Adam - Purification.
 200ml 200ml culture in Buffered rich medium.

Induced with 1mM IPTG / 5 hrs. at 30°C

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